ULTRASTRUCTURE OF *MASTIGOCLADOPSIS REPENS* (STIGONEMATALES, CYANOPHYCEAE)

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RÉSUMÉ. - L'ultrastructure d'une cyanophycée filamenteuse Stigonématale, *Mastigocladopsis repens* Hernandez-Mariné, Fernandez et Merino est décrite. Le schéma des cellules végétatives ressemble à celui des autres membres du groupe déjà étudiés. Les ramifications véritables, sortant du filament rampant, sont en T ou en Y, selon l'inclinaison de la cloison. Les hétérocystes mûrs ont une double enveloppe externe, homogène et laminaire, bien développée. On compare certains aspects avec d'autres genres.

ABSTRACT. - The fine structure of *Mastigocladopsis repens* Hernandez-Mariné, Fernandez et Merino, a filamentous Stigonematalean cyanophyte, is described. The scheme of vegetative cells is similar to others members of the group. The slope of the not transversal division septum in the main filament produces either true T-branching or Y-branching. The mature heterocysts present a well-developed homogeneous extra wall and laminated layer. Comparison with some features of other genus are presented.

RESUMEN. - Se describe la ultrastructura de *Mastigocladopsis repens* Hernandez-Mariné, Fernandez et Merino, una cianofícea filamentosa Stigonematal. El esquema de las células vegetativas es similar al de otros miembros del grupo estudiados previamente. En el filamento principal los tabiques no son nunca transversales y se forman ramificaciones en T o en Y, dependiendo de su inclinación. Los heterocistes maduros tienen exteriormente una capa homogénea y otra laminada, bien desarrolladas. Se comparan algunos aspectos con los de otros géneros.

Key-words. - Fine structure, Cyanophyceae, Stigonematales, *Mastigocladopsis repens*.

INTRODUCTION

Mastigocladopsis repens Hernandez-Mariné, Fernandez et Mérino is a filamentous Stigonematalean cyanophyte, isolated from the surface of calcareous Spanish soils (Hernández-Mariné *et al.*, 1992). The main characteristics are: trichomes sheathed with both reverse 'Y' branching and simple lateral true branching; trichomes having a single row of cells and heterocysts intercalary, lateral or terminal.

During the present study its ultrastructural features have been investigated and compared with some related previously studied Cyanophyceae, with the aim of finding coincident and discriminative characters.

MATERIALS AND METHODS

An aliquot of the same sample used for the description, (Hernández-Mariné *et al.*, 1992), was utilized to start cultures on 1% agarized BBM medium (Bold & Wynne, 1985) supplied with 1% CO_3Ca. Filaments of two weeks culture were scraped from the surface of the agar and fixed in a mixture of glutaraldehyde (2.5%) / paraformaldehyde (2%) in 0.1 M cacodylate buffer (pH=7.2) for two hours, washed several times in buffer and postfixed in 1% osmium tetroxide in the same buffer. They were dehydrated by a graded acetone series, embedded in Spurr's resin (AG, Fluka). The sections were stained with 2% uranyl acetate and lead citrate as described by Reynolds (1963). Observations were carried out in a Philips 200 transmission electron microscope.

RESULTS

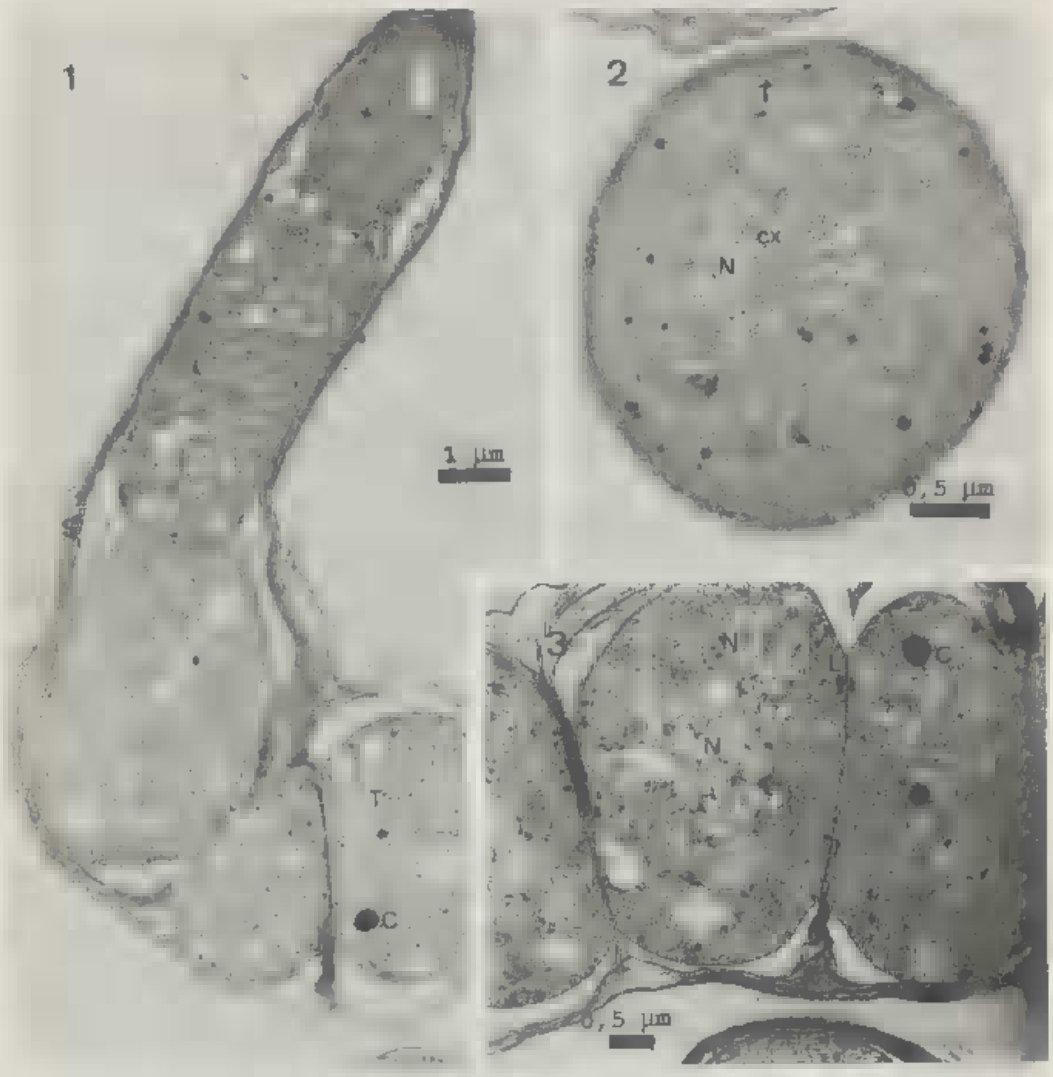
Light Microscopy

From light microscopic observations it is established (Hernández-Mariné *et al.*, 1992) that the thallus is composed of richly-branched filaments formed by two morphologically different types of cells. The main filament is built of large, pleiomorphic, barrel-shaped or rounded cells, 5 to 12 µm wide, length-width ratio 0.3 to 1.4, while the other cells, which compose the erect lateral branches and tips of the main filament, consisted of nearly uniform, long, narrow cylindrical cells, 3.0 to 5.5 µm broad and 5 to 13.5 µm long, length-width ratio 1 to 3.9. The terminal cells of the branches divide transversally. Those on the main filament, when they grow to fill the space, divide in a plane that is not transversal to the filament, giving T or Y branches (Anagnostidis & Komarek, 1990) depending on the slope of the division septum. Heterocysts are shaped like near vegetative cells, either intercalary, 4 to 16 µm long in the branches and intercalary or lateral in the pleiomorphic main filament, in the later case the shape is subconical to spherical, 5-12 µm in diameter. Motile hormogonia are formed from the narrow lateral branches and the main filament tips by breaking away from the parental trichoma.

Electron Microscopy

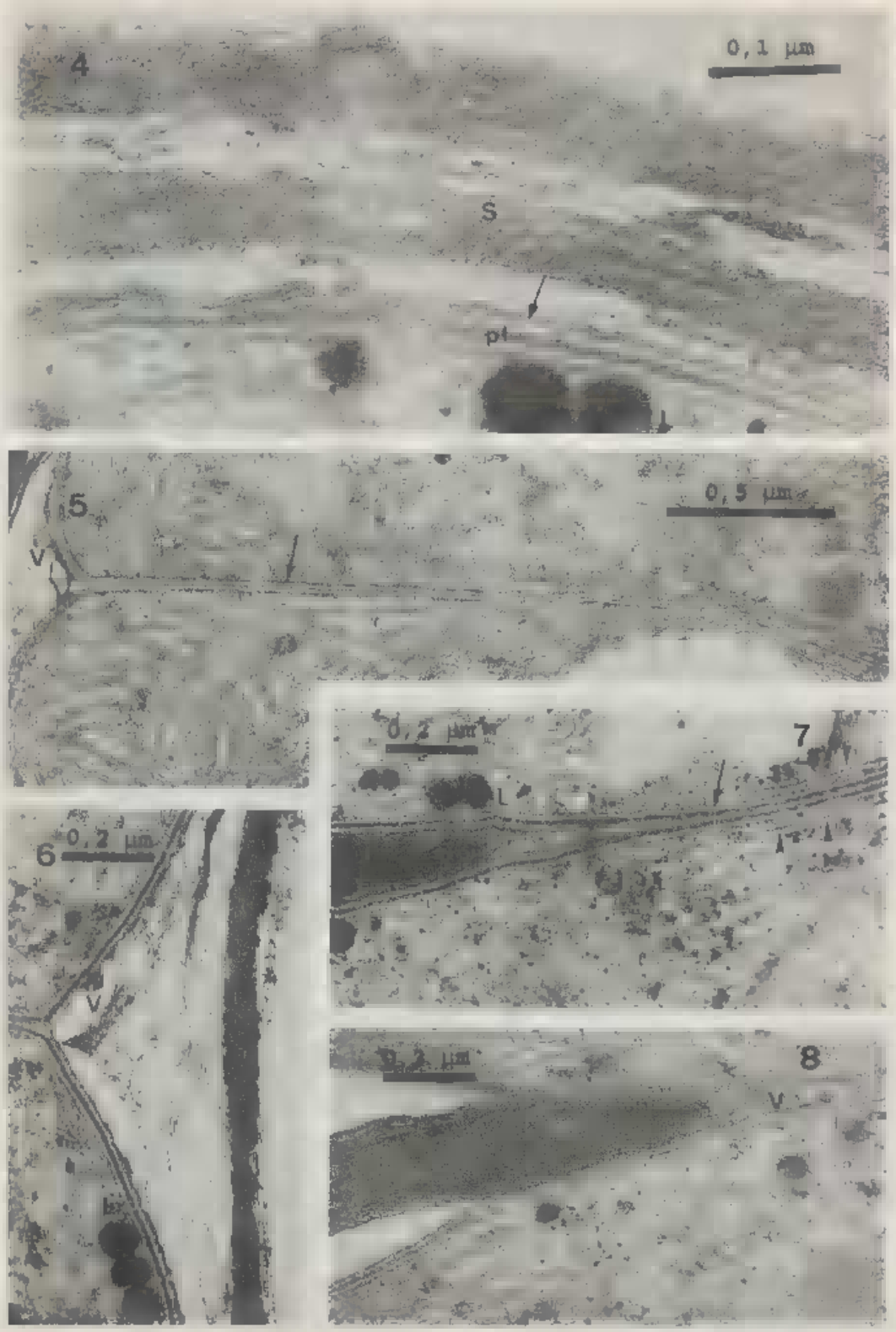
The general organization is similar to that found in other Stigonematales (Anagnostidis & Komárek, 1990) (Figs. 1 to 3).

The sheath is formed by layers of different electron density, those more electron dense are formed by microfibrils parallel to the cell wall whereas the clears layers had less compact, irregularly arranged microfibrils (Fig. 4). The first formed, most external layer, is continuous contributing to the maintenance of the thallus structure. The cell



Figs. 1-3. *Mastigocladopsis repens*, TEM micrographs. Fig. 1: Longitudinal section in the transition region between in the branches and the main filament. Fig. 2: Transverse section of a cell from the tip of the branches. Note the more or less central nucleoplasm region (N) and the thylakoids (T) arranged in whorled parallel groups. Fig. 3: Transverse section of cells from the main filament. Note the spread of the nucleoplasm region (N) and the vacuolized thylakoids.

wall (Fig. 4) is formed by a peptidoglican layer of ca. 12 nm and an outer membrane that forms vesicles migrating to the forming sheath, specially at the septum level (Figs. 5, 6). The cell division is of the septum type (Drews & Weckesser, 1982), formed by the invagination of the cytoplasmic membrane and the peptidoglican layer (Figs. 5, 6). The septum is traversed by thin plasmodesmata (Fig. 7) and at both sides can be observed a semicontinuous unit membrane lamellar sheath (Berner & Jensen 1982) separated from cytoplasmic membrane ca. 20 nm (Fig. 7), between them circular profiles and



granules are visible. When the branche-cell grows, becoming main filament, the septum region increases in width less than the rest of the cell, giving the torulose aspect (Figs. 1 to 3). The invagination of the outer membrane follows and the septum splits away, while vesicles are formed, increasing the sheath in between the cells (Figs. 6 to 8). The daughter cells are gradually separated by the insert; all states of the separation process can be seen in the main filament.

The nucleoplasm region is more or less central (Figs. 2, 3) and fingering throught the whole cell, since in the thin sections fibrils and carboxisomes can be seen everywhere, surrounded by the dense thylakoidal system with the thylakoids arranged in small curved or whorled parallel groups. Thiéry (1967) staining reveals the polyglucosic nature of the structures (Figs. 9, 10); glycogen granules are isolated among the thylakoids or in small groups, whereas different types of membranes, thylakoidal, cytoplasmatic, outer layer and the vesicles forming the sheath are manifested. Main filament cells (Figs. 1, 3) are larger and rounder than branch cells (Figs. 1, 3) and the nucleoplasm region spreads, even near the cytoplasmatic membrane. Thylakoids are vacuolized and some cell inclusions (Jensen, 1985) increases as do glycogen granules, cyanophycin granules and transparent phosphorous reserve spaces. The oblique dividing cells in the main filament have the same internal features as the neighbouring cells (Figs. 11 to 13).

The division figures are transversal in the tip of the branches and more or less oblique in the main filament, the little occurrence of the division figures suggests that division occurs only in few or one tip cells. The division more or less parallel to the main filament (Fig. 11) give T branches or lateral heterocysts. The Y branching begins by an oblique division in a cell of the main filament (Fig.12), the division products grow and one of them is displaced upwards (Figs. 13 14), and subsequently grows out as a branch with the next septum in a plane parallel to the original main filament (Fig. 15). The other division product at the base does not resume growth althought it helps to straighten the branch (Figs. 14, 15), together with the neighbourg contact cell. Persistent central regions of septum or "pit connections" were not observed.

The heterocysts are distributed either in branches (Fig. 16) without clear spacing pattern or, usually, closed to the neighbouring cell at the base of the branch in the main filament (Figs. 11, 14). Independent of the situation of the proheterocyst, when developping into a heterocyst the cells modified shape or size only slightly. The differentiation stages begin by the formation of a homogeneous extra wall layer (Lang & Fay, 1971; Nierzwicki-Bauer *et al.*, 1984 a, 1984 b) and the development of necks connecting to adjacent cells (Fig. 17) with the peptidoglycan layer traversed by

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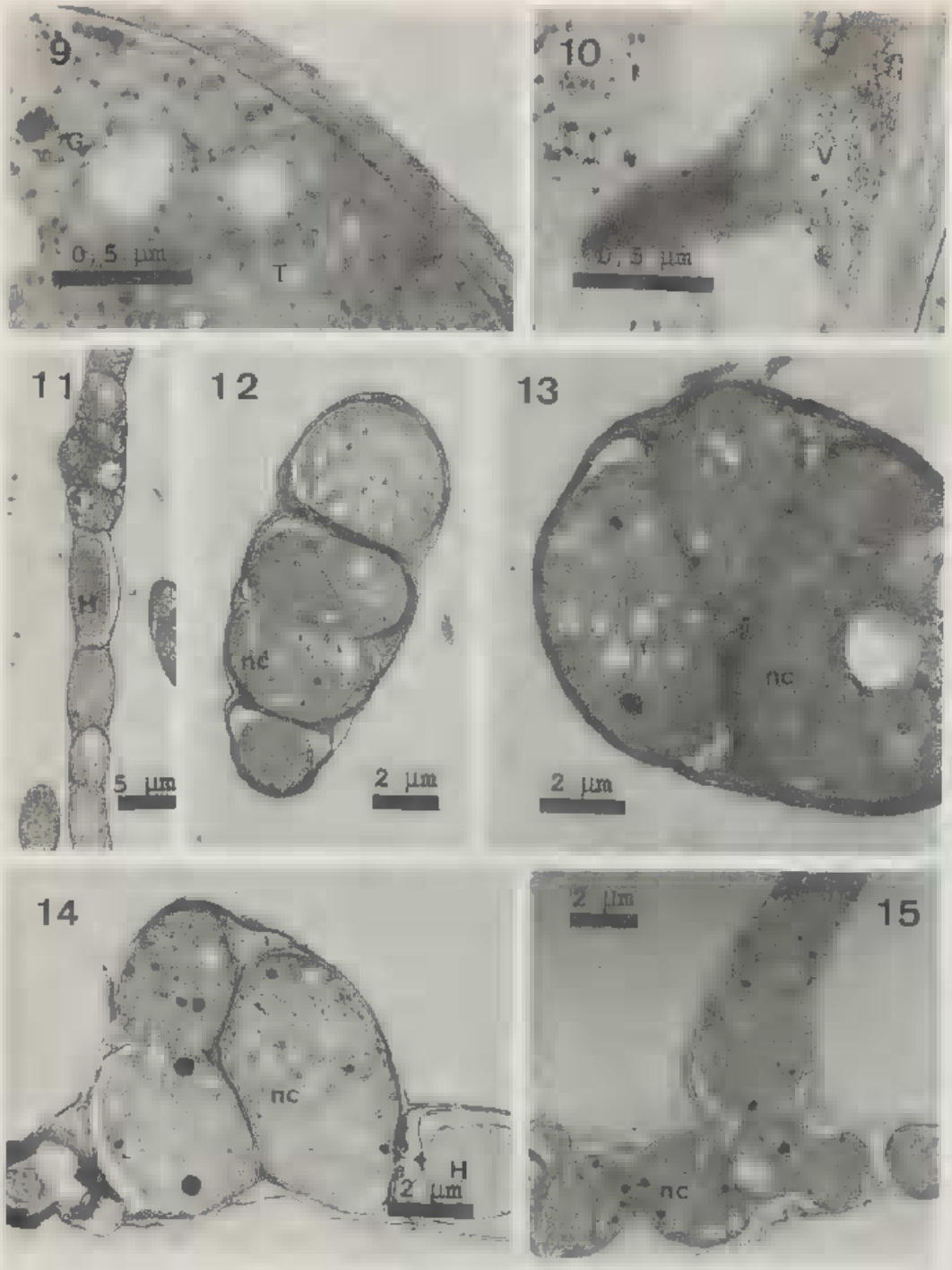
Figs. 4-8. *Mastigocladopsis repens*. TEM micrographs. Fig. 4: Substructure of the sheath and the cell wall; note pores (arrow) in the outer layer. Fig. 5: Longitudinal section through a branch filament with septum formation. Only the cytoplasmic membrane and the peptidoglican layer are involved. Fig. 6: Longitudinal section showing the transverse septum and the sheath with vesicles appearing as round profiles. Fig. 7: Splitting of the septum and invagination of the outer membrane. Note plasmodesmata through the septum (arrow) and a semicontinuous unit membrane lamellar sheath (head arrows). Fig. 7: Ingrowth of the sheath by outer wall vesicles.



Figs. 9-15. *Mastigocladopsis repens*, TEM micrographs. Figs. 9 and 10: Thiéry dying for polysaccharides showing glycogen granules, isolated or in small groups, and vesicles forming the sheath. Fig. 11: Longitudinal section. A not mature heterocyst and ■ division figure, more or less parallel to the main filament can be observed. Fig. 12: Longitudinal section showing an oblique division in a cell of the main filament, starting point of ■ Y-branching. Figs. 13-14: The oblique division products enlarges displacing up one of them. The neighbourg cell (nc) helps in the straighten of the branche. Fig. 15: Final aspect of the Y-branching.

plasmodesmata (Fig. 18). The homogeneous wall layer (Figs 16 to 19) is a thick structure and the most evident layer, first formed around the neck, where it is broader and surrounds all the heterocyst. The fibrous wall layer was not observed, possibly because it was so close to the sheath that discrimination was impossible. The laminated inner layer is the last formed. It begins its development near the neck and continues increasing towards the sides (Figs. 17, 20, 21). It is thin and, at least near the neck, more electron dense than the other layers. Growth of the heterocyst laminated layer is helped by migrating vesicles from the cell wall outer layer (Figs. 20, 21). Heterocyst internal differentiation is slowed down and involves gradual lost of granular reserves, degradation of carboxisomes and thylakoid wrinkle and damage (Figs. 19 to 21). There are no closely packed intracytoplasmic membranes at the poles at any time but cyanophycin-like material plugs. The interior of mature heterocyst is rather homogeneous and of not high electron density (Figs 14, 16).

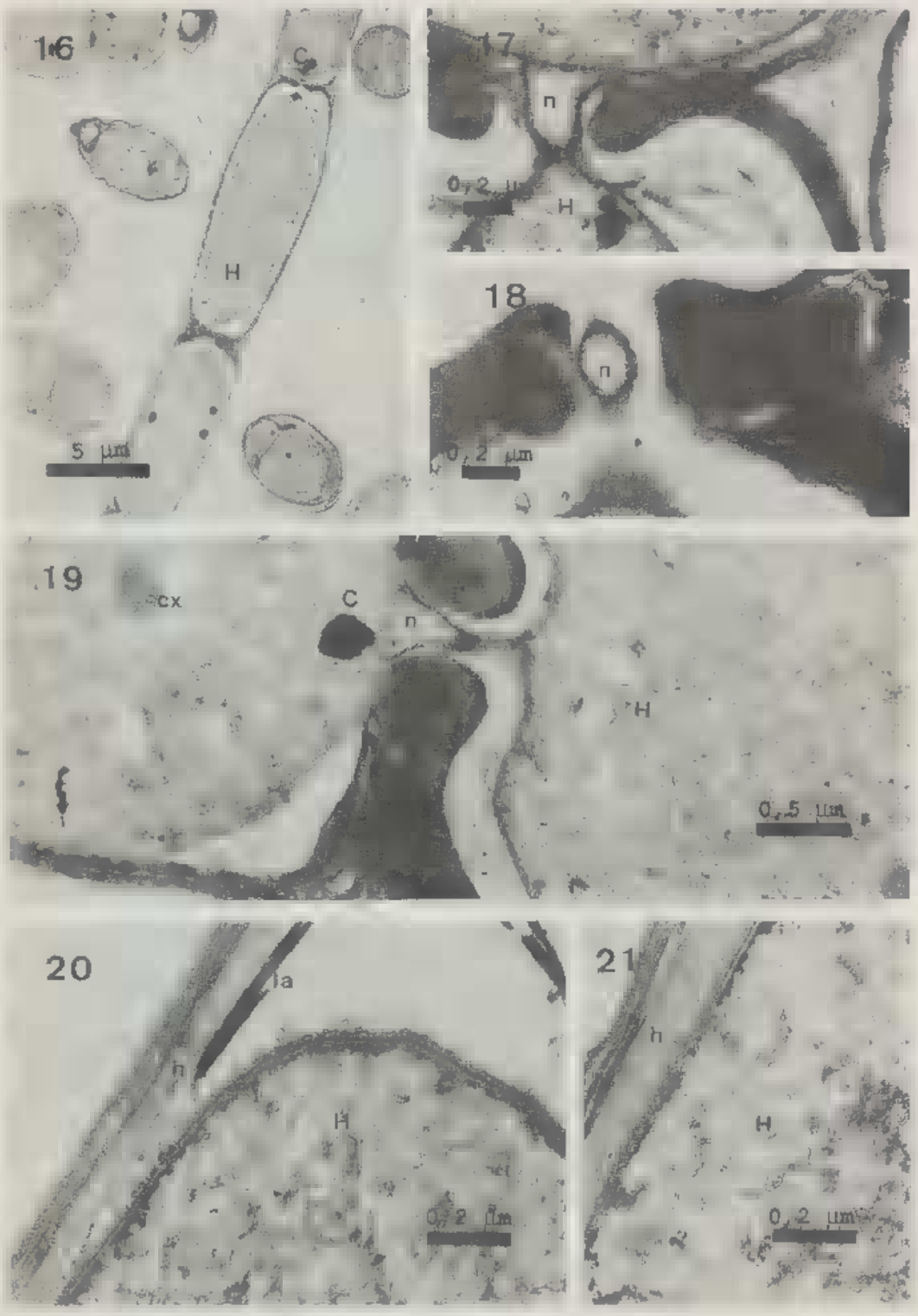
DISCUSSION

The genus was described by Iyengar & Desikachary (1946) who assigned it to a new family Mastigocladopsidaceae. It was later transfered (Anagnostidis & Komarek, 1990) to the Nostochopsaceae, enlarging the diagnostic features because the reverse 'Y' branching is present in *Mastigocladopsis* and not in the others members of the Nostochopsaceae

The morphological and ultrastructural characteristics of *M. repens* are consistent with those of previously described Stigonematales (Thurston & Ingram, 1971; Butler & Allsop, 1972; Rippka *et al.*, 1979; Nierzwicki *et al.*, 1982; Anagnostidis & Komarek, 1990) and specially with the extensively studied *Mastigocladus laminosus* Cohn (Nierzwicki *et al.*, 1982; Balkwill *et al.*, 1984; Nierzwicki-Bauer *et al.*, 1984). The thickness of the sheath and wide peptidoglycan layer are adaptations to water stress (Campbell, 1979; Jensen, 1982; Winder, 1991). Living on soil *M. repens* presents a moderate thick sheath and a 'normal' peptidoglycan layer indicating its habitat is not an extreme environment.

The ultrastructure of T lateral branching is basically the same ■ that described for *Fischerella ambigua* (Thurston & Ingram, 1971) and *M. laminosus* (Nierzwicki *et al.*, 1982; Balkwill *et al.*, 1984). The formation of Y-branches has not been described at the ultrastructural level in *Mastigocladus laminosus* although it has been reported at the optical level (Desikachary, 1959, Plate 128, Figs. 1 to 6). Balkwill *et al.* (1984) reported that diagonal division occurred in the main filament after branch formation and neither of the daughter cells divided subsequently, whereas in *M. repens* one of the growing daughters forms the 'Y' branche.

The process of sheath formation by vesicles (Thurston & Ingram, 1971; Butler & Allsopp, 1972) is also present in the formation of the heterocyst external layers. The ultrastructural changes taking place during heterocyst differentiation in *M. laminosus* were compared by Nierzwicki-Bauer *et al.*, (1984 b) with those in *Anabaena* ssp., they reported differences in the sequence of differentiation and final aspect. The heterocysts in *M. repens* differs from both in lacking closely packed intracytoplasmic membranes.



Figs. 16-21. *Mastigocladopsis repens*, TEM micrographs. Fig. 16: Longitudinal section of a mature heterocyst. Fig. 17: Detail of the neck area in longitudinal section. Fig. 18: Detail of the neck area in tangencial longitudinal section. Note plasmodesmata. Fig. 19: Detail of morphological characteristics of the cytoplasm of an heterocyst and the adjacent vegetative cell. Note cyanophycin-like plug (C) and the thick homogeneous extra wall layer. Figs. 20-21. Enlarged sections of the not mature heterocyst in Fig. 11. Note the vesicles and the forming laminated layer.

The sequence of differentiation, the thick homogeneous wall layer and the presence of the laminated layer are the features shared by *M. repens* and *Anabaena* ssp. heterocysts (Lang & Fay, 1971; Adams & Carr, 1981), whereas they are the distinction from those in *M. laminosus* (Nierzwicki-Bauer *et al.*, 1984 a, 1984 b). In comparing structures the possible effect of different grown medium (Lang *et al.*, 1987) or fixation procedures must be borne in mind. Lang & Fay (1971) found that the laminated layer was preserved well only with a glutaraldehyde-$KMnO_4$, fixation that has neither been used in this study nor by others (Nierzwicki-Bauer *et al.*, 1984 a, 1984 b; Couté & Bury, 1988).

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